(Eta6-arene) ruthenium(II) complexes and metallo-papain hybrid as Lewis acid catalysts of Diels-Alder reaction in water.

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(η^6-Arene) ruthenium(II) complexes and metallo-papain hybrid as Lewis acid catalysts of Diels–Alder reaction in water†

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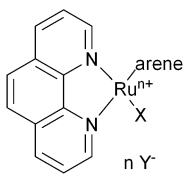
Received 25th January 2010, Accepted 22nd March 2010
First published as an Advance Article on the web 20th April 2010
DOI: 10.1039/c001630f

Covalent embedding of a (η^6-arene) ruthenium(II) complex into the protein papain gives rise to a metalloenzyme displaying a catalytic efficiency for a Lewis acid-mediated catalysed Diels–Alder reaction enhanced by two orders of magnitude in water.

Artificial metalloenzymes combining transition metal species and biopolymers have attracted much attention during the last five years as asymmetric catalysts operating under mild conditions.[1] Artificial Diels–Alderases resulting from the association of Cu(II) ion with multi-dentate nitrogen ligands strongly interacting with or covalently linked to DNA or albumins have been reported.[2,3,4] In these examples, the metal complex acted as a Lewis acid coordinating the heteroatoms of the dienophile thus lowering the energy of its LUMO.[5] This very attractive approach is nonetheless limited to two-point binding dienophile substrates that can chelate to the Lewis acid.[4,6] Several Lewis acids have been shown to accelerate Diels–Alder (D–A) reactions of one-point binding dienophiles in water.[7] However, the construction of artificial metalloenzymes from these Lewis acids is not straightforward because none of them can be readily associated with proteins.

Organometallic ruthenium(II) complexes, in particular [(η^6-arene)$RuL_2(H_2O)]^{2+}$ with L_2 being a bidentate P,P-, P,N-, N,O- or N,N-ligand have been shown to catalyse D–A reactions in organic solvents thanks to their Lewis acid character.[8,9] Catalysis involves transient coordination of the dienophile carbonyl group to the metal by displacement of a coordinated solvent molecule.[9] As these complexes are air- and water-tolerant, we reasoned that they should be suitable to construct artificial Diels–Alderases through their association with a protein host. We chose the cysteine endoproteinase papain (PAP) for this purpose because this protein conveniently contains a single free cysteine residue to which complexes may be anchored by covalent attachment *via* one of the ligands coordinating the metal, allowing a precise positioning of the complexes with respect to the protein host, *i.e.* within its catalytic pocket.[10,11] Historically, the first semi-synthetic enzymes were designed by Kaiser *et al.* from this protein.[12,13] More recently, several metal complexes[14] and a pyridoxamine cofactor[15] were attached to papain for the same purpose.

We synthesized complex **1a** (Scheme 1) according to our previously described procedure.[11] This complex carried a chloroacetamide functional group on the arene ligand for covalent anchoring to papain. Crystals of **1a** were obtained when the Cl^- counter-anion was replaced by BF_4^-. The molecular structure of this molecule was solved by X-ray diffraction (Fig. 1). It shows a three-legged piano stool structure typical of these complexes. The dicationic aquo complex **1b** was produced by chloride abstraction in the presence of Ag^+ ions. Furthermore, the non-functionalized monocationic (**2a** and **3a**) and dicationic (**2b** and **3b**) complexes were prepared as models for the catalytic studies.



arene	X=Cl, Y=Cl, n=1	X=H_2O, Y=BF_4, n=2
$Ph(CH_2)_2NHCOCH_2Cl$	**1a**	**1b**
p-cymene	**2a**	**2b**
benzene	**3a**	**3b**

Scheme 1 Numbered complexes.

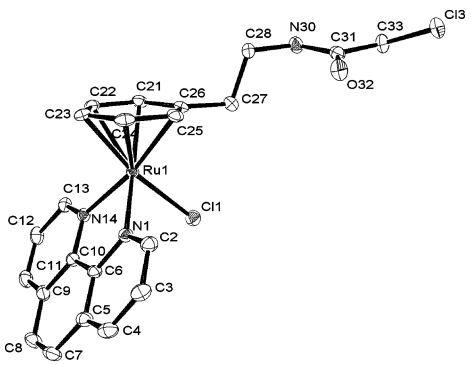


Fig. 1 ORTEP drawing of the molecular structure of **1a**. Ellipsoids are shown at 50% probability.

The D–A reaction between cyclopentadiene and acrolein was chosen as a test reaction to evaluate the catalytic ability of the complexes (and the metalloenzymes) in water. The formation of

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† Electronic supplementary information (ESI) available: Synthetical procedures, X-ray crystallographic data of **1a**·BF_4, kinetics data of aquation of **1a** and anation of **1b** and of Diels–Alder reactions. CCDC reference number 742394. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c001630f

Table 1 Kinetic parameters for the reaction of CpH (0.46 M) with acrolein (0.046 M) in water at 2 °C

Entry	Catalyst	$k_{obs}/10^3$ min^{-1} [a]	Yield (%)[b]	TOF/h^{-1} [c]
1	No catalyst	5.1 ± 1.6	62	—
2	10 mol% **1a**	10 ± 2	61	1.4
3	10 mol% **1b**	17.0 ± 0.7	65	3.6
4	10 mol% **2b**	17.4 ± 1.4	65	3.4
5	10 mol% **3b**	15.1 ± 1.6	68	3.4
6	0.2 mol% **1a**	4.8 ± 1.0	62	20
7	0.2 mol% PAP-NEM	19.0 ± 1.3	50	130
8	0.2 mol% **1a** + 0.2 mol% PAP-NEM	15.8 ± 1.3	54	65
9	0.2 mol% PAP-**1a**	14.6 ± 1.4	88	220

[a] Pseudo-first order rate constant calculated from the non-linear regression analysis of the kinetic plots (see ESI†). [b] Determined by GC after 250 min using decane as an internal standard. [c] Turnover frequency calculated at 30 min reaction time after subtraction of product contribution resulting from uncatalysed reaction.

the *endo*- and *exo*- cycloaddition adducts was monitored during 250 to 500 min by chiral GC analysis. The pseudo-first-order rate constants of reaction k_{obs} were extracted from the non-linear regression analysis of the time plots (Table 1, Fig. S6†).

In the absence of ruthenium species (entry 1, Table 1), the D–A reaction went to 62% conversion in 250 min with a rate constant of 0.005 min^{-1} and the *endo*/*exo* ratio was equal to 92/8. It was anticipated that this reaction should take place in these conditions, owing to the well-described rate increase observed in aqueous medium for cycloaddition reactions.[16,17] When 10 mol% (with respect to the dienophile) of the dicationic complexes **1b–3b** were added to the reaction medium (entries 3 to 5), an increase of the reaction rate by a factor of *ca.* 3 was observed. The yield in bicyclo[2.2.1]hept-5-ene-2-carboxaldehyde after 250 min together with the *endo*/*exo* ratio were identical to the experiment carried out without Ru species. ^{1}H NMR monitoring of the reaction in D_2O at r.t. confirmed the formation of bicyclo[2.2.1]hept-5-ene-2-carboxaldehyde together with dicyclopentadiene. Although the tolerance of a salen Ru(II) Lewis acid activity to the presence of water has been previously mentioned,[18] the ability of (η^6-arene)Ru(II) Lewis acids to catalyse a Diels–Alder reaction in pure water is demonstrated for the first time.

More surprisingly, cycloaddition between CpH and acrolein was also accelerated in the presence of **1a** although this complex is not a Lewis acid (entry 2). This may be explained by the aquation reaction undergone by **1a** in aqueous medium in the same timescale, allowing *in situ* generation of the catalytic species **1b**.[19]‡ The lower efficiency of **1a** compared to **1b** is due to a low amount of catalytic species at the early stages of the reaction.

Affinity-purified papain was allowed to react with **1a** in the presence of 0.15 M NaCl (to avoid aquation at this stage). Chemoselective attachment of **1a** to papain's single cysteine residue (Cys25) was evidenced by a complete loss of hydrolytic activity of the enzyme upon reaction. ESI-MS analysis of the hybrid protein gave an observed molecular mass of 23 914 ± 4, corresponding to the adduct resulting from nucleophilic substitution at the chloroacetamide function of **1a** and replacement of the ancillary chloro ligand by a formato ligand during the MS analysis (Fig. S2†). The metalloprotein was then tested as a catalyst in the Diels–Alder reaction between acrolein and CpH at an initial loading of 1.6 mol% with respect to dienophile. The time plot showed that the reaction was completed in *ca.* 120 min and the yield reached 90%. More interestingly, the same result was obtained with 0.8 and even 0.2 mol% metalloprotein (Fig. 2). By comparison, it took 360 min to reach completion without catalyst and 250 min with the complexes alone (Fig. S6). The TOF was equal to 220 h^{-1} at 30 min (entry 9). This is an improvement by a factor of 60 with respect to the dicationic complexes **1b–3b** alone and 150 when related to the monocationic complex **1a** at 10 mol%. Notice that no enantiomeric excess was detected.

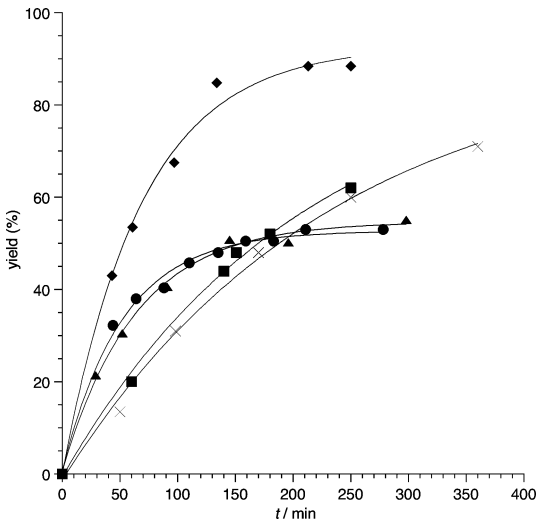

Fig. 2 Kinetic plots of the Diels–Alder reaction between CpH and acrolein in the absence (×) and presence of **1a**, (■), PAP-NEM (●), **1a** + PAP-NEM (▲) and PAP-**1a** (♦) at 0.2 mol%. Curves result from non-linear regression analysis of the data.

To delineate the mechanism by which the metalloenzyme catalysed the D–A reaction, additional experiments were run in parallel with **1a** and papain reacted with the thiol-blocking agent NEM (PAP-NEM). It appeared that **1a** alone had no significant effect on the rate and final yield at 0.2 mol% loading with respect to control (Fig. 2 and Table 1, entry 6). Conversely, PAP-NEM at 0.2 mol% loading led to an increase of the reaction rate (k_{obs} multiplied by 4, entry 7) with respect to control but had a detrimental effect on the yield that reached a maximum of only 50% after 200 min. This initial rate enhancement could be due to a non covalent catalytic effect occurring by binding of the diene and/or the dienophile to a protein cavity in a similar fashion as cyclodextrins operate.[17,20] This process could affect the rate of both cycloaddition reactions previously shown to take place by ^{1}H NMR. Alternatively, it could be due to covalent catalysis, the carbonyl dienophile being activated by reversible formation of iminium adduct resulting from the condensation with the protein's amine groups, just as amines were shown to catalyse D–A reactions.[21] Finally, addition of both **1a** and PAP-NEM at 0.2 mol% to the reaction mixture gave the same trend as PAP-NEM regarding the yield and the rate (entry 8). Thus, only covalent binding of **1a** to papain led to an efficient catalyst at low loading. The high conversion rate measured in this case may be due to the fact that the catalyst only accelerated the cycloaddition of CpH and acrolein. The partners taken separately or not covalently combined had low or even no catalytic effect at the same loading on the reaction of acrolein and CpH in water.

Reaction rate accelerations were previously observed for artificial metalloenzymes resulting from the supramolecular or covalent anchoring of metal complexes to biopolymers as compared to the metal cofactors alone.[3,22] Flavopapains were also more efficient catalysts than the flavin analogues alone.[12] In the present case, the enhanced catalytic activity of the metal complex embedded within the protein may be due to an increase of its Lewis acid character owing to the confining of the metallic entity that in turn activates the carbonyl group of the dienophile in a more efficient manner.

In conclusion, covalent anchoring of an (η^6-arene) ruthenium(II) complex to the cysteine endoproteinase papain yielded an artificial metalloenzyme with a turnover frequency on a Diels–Alder increased by two orders of magnitude with respect to the complex alone.

Notes and references

‡ The first-order rate constant of hydrolysis of **1a** was equal to 0.0088 min^{-1} at room temperature, giving a half-life of 79 min (see ESI†).

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